Amphetamine-Antagonistic Property of 4-Phenyltetrahydroisoquinoline: Effect on Noradrenaline Release in Spinal Cord Slices

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ABSTRACT—The amphetamine-related compounds methamphetamine, phenylethylamine and nomifensine increased the K^+ -evoked release of endogenous noradrenaline from rat spinal cord slices. 4-Phenyl-1,2,3,4-tetrahydroisoquinoline (4PTIQ), which alone did not affect the K^+ -evoked release of noradrenaline, inhibited the noradrenaline-releasing effects of methamphetamine, phenylethylamine and nomifensine. 4PTIQ revealed a weak noradrenaline-uptake inhibitory effect, and the effect was weaker than those of desipramine and nomifensine. These results showed that 4PTIQ is an antagonist against the amine-releasing effects of amphetamines.

Keywords: 4-Phenyl-1,2,3,4-tetrahydroisoquinoline, Methamphetamine, Noradrenaline release, Spinal cord

The CNS-stimulant actions of amphetamines are considered to be due to their ability to release monoamines from monoaminergic nerve terminals (1, 2), inhibition of monoamine uptake (3) and inhibition of monoamine oxidase (MAO) (4). However, the mechanism of the CNS-stimulating action of amphetamines, especially their monoamine-releasing effect, is still unclear. The most widely accepted hypothesis is that amphetamine increases amine efflux by accelerated exchange diffusion (5, 6). By recording the spinal monosynaptic reflex (MSR) of spinalized rats, we have found that amphetamine-related compounds, methamphetamine, phenylethylamine and nomifensine (8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline), increase the amplitude of the MSR by releasing noradrenaline from the noradrenergic terminals of descending fibers (7–10), and have suggested that 4-phenyl-1,2,3,4-tetrahydroisoquinoline (4PTIQ) antagonizes the effects of these compounds by inhibiting their noradrenaline-releasing effects (9).

In the present study, to further elucidate the inhibitory effects of 4PTIQ on noradrenaline release, we studied effects of 4PTIQ on the release of endogenous noradrenaline in rat spinal cord slices. The amphetamine-

amine-related compounds methamphetamine, phenylethylamine and nomifensine facilitated the K^+ -evoked release of noradrenaline, and 4PTIQ inhibited the stimulatory effects of amphetamines on the release. The blocking effects of 4PTIQ and other compounds on the noradrenaline uptake were also evaluated.

MATERIALS AND METHODS

Perfusion of rat spinal cord slices

Male Wistar rats, aged 9–10 weeks (b.wt. 250–330 g), were sacrificed by decapitation. The lumbar enlargement of the spinal cord (L4–L5) was rapidly removed. The tissue was chopped into $300 \times 300\text{-}\mu\text{m}$ slices with a MacIlwain tissue chopper and then dispersed in Krebs-Ringer bicarbonate buffer of the following composition: 118 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl_2 , 1.2 mM MgCl_2 , 1.0 mM NaH_2PO_4 , 25 mM NaHCO_3 , 11.1 mM glucose, 0.004 mM EDTA and 0.11 mM ascorbic acid. High K^+ solution was made by replacing equimolar Na^+ . The medium, pH 7.4, was gassed throughout the experiment with 95% O_2 –5% CO_2 . Slices of about 50- to 60-mg wet tissue weight were placed in a perfusion chamber (0.5-ml internal volume) and perfused with the medium (37°C) for 60 min at a rate of 0.5 ml/min. A single 8-min (4-ml) sample was

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collected.

Measurement of noradrenaline

Noradrenaline in the perfusate was concentrated using alumina and determined by high performance liquid chromatography (HPLC) and electrochemical detection (ECD). The detection limit for noradrenaline was 50 fmol. Noradrenaline release was determined with 30 mM high K^+ at 76 (S_1) and 176 (S_2) min after the start of perfusion. The release of noradrenaline evoked by high- K^+ stimulation was estimated as described by Kamal et al. (2). Evoked release at the S_1 and S_2 periods of stimulation was estimated from the total amount minus basal release during stimulation. The test drugs were added to the perfusion medium 8 min before S_2 and during stimulation, and their effects were evaluated in terms of the ratio of the amounts evoked in the S_1 and S_2 periods of stimulation (S_2/S_1).

[3H]Noradrenaline uptake

The [3H]noradrenaline uptake experiment was performed with synaptosomal fractions of rat spinal cord. The lumbar segment of the spinal cord was isolated, weighed and homogenized gently in 10 volumes of 0.32 M sucrose in a smooth glass homogenizer with a Teflon pestle (0.1- to 0.15-mm clearance). Following centrifugation of the homogenate at 4°C for 10 min at $1000 \times g$, the supernatant fraction was centrifuged at $12,000 \times g$ for 30 min. The crude synaptosomal pellet (P_2) was gently resuspended in 5–10 volumes of Krebs-Ringer bicarbonate buffer. Uptake of ($-$)[3H]noradrenaline was allowed to proceed for 5 min at 37°C, in 1 ml (final volume) of incubation medium that had been prewarmed for 10 min at 37°C prior to the addition of synaptosomes. The medium contained pargyline ($10 \mu M$) as a MAO inhibitor. ($-$)[3H]Noradrenaline, 13.7 Ci/mmol, was diluted, and the final concentration of ($-$)[3H]noradrenaline was 35 nM. Following incubation at 0°C and 37°C for 5 min, the samples were diluted with 5 ml of chilled 50 mM Tris-HCl buffer (pH 7.4), filtered under vacuum on a Whatman GF/B manifold and then washed with a further 5 ml of buffer. Prior to filtration, the filters had been soaked with polyethyleneimine (0.1%) to lower the adsorption of ($-$)[3H]noradrenaline to the filters. Radioactivity on the filters was counted in 5 ml of Triton X-100 in toluene, containing 2,5-diphenyloxazol (PPO) and 1,4-di[2-(5-phenyloxazolyl)]benzene (POPOP). All determinations were carried out in duplicate.

Statistical analyses

Numerical results are expressed as mean $\pm$ S.E., and Duncan's New Multiple Range test was used to calcu-

late statistical significance where appropriate. A P value less than 0.05 was considered to indicate a significant difference.

Drugs

Drugs used were ($-$)-noradrenaline hydrochloride (Alterenol, Sigma), pargyline hydrochloride (Sigma), 2-phenylethylamine hydrochloride (Tokyo Kasei), desipramine hydrochloride (Ciba-Geigy), $S(+)$ -methamphetamine hydrochloride (Dainippon Seiyaku), nomifensine maleate (Hoechst), ($-$)[3H]noradrenaline (specific activity, 13.7 Ci/mmol; New England Nuclear) and 4-phenyl-1,2,3,4-tetrahydroisoquinoline (4PTIQ) hydrochloride. 4PTIQ hydrochloride was synthesized (mp. of 217–219°C), and its purity was more than 99.7% by elemental analysis.

RESULTS

Effects of amphetamine-related compounds on K^+ -evoked release of endogenous noradrenaline

In the control experiment, the releases induced by 30 mM K^+ from rat spinal cord slices, S_1 and S_2 , were 1.41 ± 0.22 and 1.11 ± 0.23 pg/mg/min ($n = 10$), respectively. This high- K^+ -evoked release of endogenous noradrenaline was Ca^{2+} -dependent.

Exposure of the spinal cord to methamphetamine (3×10^{-6} M), phenylethylamine (10^{-5} M) or nomifensine (10^{-5} M) increased the S_2 (Figs. 1C and 2) without affecting the spontaneous outflow of noradrenaline. The ratio S_2/S_1 in the control experiment was not significantly different from unity. However, the S_2/S_1 ratios of methamphetamine (3×10^{-6} M), phenylethylamine (10^{-5} M) and nomifensine (10^{-5} M) were 2.39 ± 0.20 , 2.62 ± 0.48 and 1.81 ± 0.31 , respectively, being significantly different from that of the control (Figs. 1C and 2). Pargyline (10^{-5} M) and deprenyl (10^{-5} M), both MAO inhibitors, and desipramine (10^{-5} M), an uptake inhibitor, did not increase the S_2/S_1 ratio (Fig. 2). 4PTIQ (10^{-5} M) alone added 20 min before S_2 did not affect the S_2/S_1 ratio (1.16 ± 0.15) (Fig. 2). 4PTIQ (10^{-5} M) added along with amphetamine-related compounds significantly reduced the S_2/S_1 ratios of methamphetamine (0.92 ± 0.11), phenylethylamine (1.18 ± 0.14) and nomifensine (0.85 ± 0.06) (Figs. 1D and 2).

Effects of amphetamine-related compounds on [3H]noradrenaline uptake into rat spinal cord synaptosomes

The inhibitory effects on [3H]noradrenaline uptake were compared among desipramine, nomifensine, methamphetamine, phenylethylamine and 4PTIQ. The order of potency in inhibiting [3H]noradrenaline uptake

Fig. 1. Effects of 4PTIQ and methamphetamine on K^+ -evoked release of endogenous noradrenaline and inhibitory effects of 4PTIQ on facilitation of K^+ -evoked noradrenaline release from rat spinal cord slices induced by methamphetamine. High- K^+ -stimulation was performed by increasing the KCl concentration of the perfusion medium to 30 mM for 8 min at 76 (S_1) and 176 (S_2) min after the start of perfusion (short line). The efflux of endogenous noradrenaline was measured by HPLC with electrochemical detection. Ordinates represent the overflow of endogenous noradrenaline in the perfusate for each 8-min sample. Abscissae represent the time after the spinal cord slices had been placed in the perfusion chamber. Drugs were applied to the perfusion medium 8 min before S_2 on the abscissae (long line). A, Control; B, 10^{-5} M 4PTIQ; C, 3×10^{-6} M methamphetamine; D, 10^{-5} M 4PTIQ + 3×10^{-6} M methamphetamine. Each value represents the mean $\pm$ S.E.M. of 3–6 experiments. #: $P < 0.05$, corresponding column.

Fig. 2. Effects of various drugs on K^+ -evoked release of endogenous noradrenaline and inhibitory effects of 4PTIQ on facilitation of K^+ -evoked noradrenaline release from rat spinal cord slices induced by stimulants. High- K^+ -stimulation was performed by increasing the KCl concentration of the perfusion medium to 30 mM for 8 min at 76 (S_1) and 176 (S_2) min after the start of perfusion (shown in Fig. 1). The ordinate represents ratios of noradrenaline released in the S_1 and S_2 periods of stimulation. CONT, control; PAR, 10^{-5} M pargyline; DEP, 10^{-5} M deprenyl; DES, 10^{-5} M desipramine; 10^{-5} M 4PTIQ; MAP, 3×10^{-6} M methamphetamine; PEA, 10^{-5} M phenylethylamine; NOM, 10^{-5} M nomifensine. Each value represents the mean $\pm$ S.E.M. of 3–6 experiments. #: $P < 0.05$ vs. control. *: $P < 0.05$ vs. corresponding column.

Table 1. Inhibition of [³H]noradrenaline uptake into synaptosomes by drugs

Drugs	[³ H]Noradrenaline uptake IC ₅₀ (−log M)
Desipramine	8.02 ± 0.07
Nomifensine	7.31 ± 0.09
Phenylethylamine	7.01 ± 0.19
Methamphetamine	6.94 ± 0.04
4PTIQ	6.10 ± 0.11

Spinal cord synaptosomes were incubated in the presence of the test drug for 10 min before the addition of [³H]noradrenaline (35 nM). Uptake was terminated after 5 min by addition of ice-cold Krebs-Ringer buffer, followed by rapid filtration. Specific uptake of [³H]noradrenaline into synaptosomes was estimated from the difference between the uptake at 37°C and that at 0°C. The IC₅₀ value is expressed as the mean ± S.E.M. of 3–5 experiments, each performed in duplicate.

was desipramine > nomifensine > phenylethylamine = methamphetamine > 4PTIQ (Table 1). 4PTIQ was thus less potent than the other compounds.

DISCUSSION

Terminals of noradrenergic neurons in the ventral horns of the spinal cord originate in the brain stem, e.g., the locus ceruleus (11–13). Methamphetamine, phenylethylamine and nomifensine release noradrenaline from the terminals of noradrenergic descending fibers, and the released noradrenaline increases the excitability of motoneurons and spinal reflex potentials via α_1 -adrenoceptors (9, 14–16). On the basis of recordings made of spinal reflex potentials, we have suggested that 4PTIQ blocks the noradrenaline-releasing effects of methamphetamine, phenylethylamine and nomifensine, and consequently antagonizes the MSR-stimulating effects of amphetamines (9).

In the present study, methamphetamine, phenylethylamine and nomifensine enhanced the K⁺-evoked release of noradrenaline. However, pargyline and deprenyl, MAO inhibitors, and desipramine, an amine uptake inhibitor, did not affect the S₂/S₁ ratio of the K⁺-evoked noradrenaline release. These results suggest that effects other than inhibition of MAO and its uptake enhanced the release of noradrenaline. Stamford et al. (17) showed that uptake inhibitors stimulate the overflow of dopamine in the nucleus accumbens during electrical stimulation of the medial forebrain bundle, without blocking the reuptake of dopamine after cessation of stimulation. They concluded that some uptake blockers can directly increase the release of dopamine in a manner unrelated to uptake blockade. Some other

studies have shown dissociation of the actions of uptake blockers on dopamine release and dopamine uptake (3, 18). The present results, showing that methamphetamine, phenylethylamine and nomifensine increased the K⁺-evoked release of noradrenaline, resemble these of Stamford et al. (17).

4PTIQ, which alone did not increase the release of noradrenaline, reduced the increase in the K⁺-evoked release of noradrenaline produced by amphetamines. Thus the present results directly demonstrated the inhibitory effects of 4PTIQ on the noradrenaline-releasing effects of amphetamines, and confirmed the results obtained in reflex experiments. Desipramine (10^{−6} M) added along with methamphetamine significantly did not affect the S₂/S₁ ratio of methamphetamine. This fundamentally supports the possibility that the inhibiting effect of 4PTIQ on the noradrenaline-releasing effects of methamphetamine is different from the noradrenaline uptake-blocking effects of uptake-blockers such as desipramine.

4PTIQ showed a weak inhibitory effect on noradrenaline uptake into spinal cord synaptosomes, and the effect was weaker than those of all the other compounds tested. Thus, the inhibitory effects of 4PTIQ on amphetamines may not be due to the uptake-blocking effects of this compound. In addition, 4PTIQ blocked the noradrenaline-releasing effect of nomifensine, which is a strong amine uptake inhibitor (19).

The present results showed that 4PTIQ antagonizes the noradrenaline-releasing effects of methamphetamine, phenylethylamine and nomifensine in spinal cord slices. Preliminary data obtained in a behavioral study showed that 4PTIQ (s.c or microinjected into the nucleus accumbens) inhibited the ambulation-stimulating effect and dopamine-releasing effects of methamphetamine measured by the microdialysis method. Thus, 4PTIQ is considered to be a compound which has antagonistic effects against amphetamines and may be a useful tool for elucidating the monoamine-releasing mechanism of amphetamines.

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